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Strong Correlation between Liver and Serum Levels of Hepatitis C Virus Core Antigen and RNA in Chronically Infected Patients

V. Descamps,[a,b] A. Op de Beeck,[c] C. Plassart,[a,b] E. Brochot,[a,b] C. François,[a,b] F. Helle,[b] M. Adler,[d] N. Bourgeois,[d] D. Degré,[d,e] G. Duverlie,[a,b] and S. Castelain[a,b]

Virology Department, Amiens University Hospital, South Hospital, Amiens, France[a]; Jules Verne University of Picardy, Amiens, France[b]; Laboratory of Molecular Virology, Medicine Faculty, Université libre de Bruxelles, Brussels, Belgium[c]; Department of Gastroenterology, Hepatopancreatology and Digestive Oncology, Erasme Hospital, Université libre de Bruxelles, Brussels, Belgium[d]; and Laboratory of Experimental Gastroenterology, Université libre de Bruxelles, Brussels, Belgium[e]

HCV core antigen (Ag) and HCV RNA levels were evaluated in matched liver biopsy samples and sera from 22 patients with hepatitis C infection by using the quantitative Architect HCV Ag immunoassay and a real-time RT-qPCR assay, respectively. The data showed a strong correlation between liver and serum compartments of HCV Ag levels ($r = 0.80$) and HCV RNA levels ($r = 0.87$). In summary, the serum HCV Ag and RNA levels reflect the intrahepatic values.

Hepatitis C virus (HCV) is an important human pathogen; worldwide, over 170 million people are chronically infected. The standard virologic diagnosis of infection is based on the detection of specific anti-HCV antibodies and cannot distinguish between past and active infections. Therefore, assays of the HCV RNA load in serum or plasma have become essential for confirming active infections and guiding the initiation and continuation of treatment with pegylated interferon and ribavirin or other antivirals. At present, real-time PCR is the benchmark technique for detecting and quantitating HCV RNA in clinical practice, with limits of detection of 10 to 15 IU/ml (4, 7, 15). However, these assays require high levels of technical skill and are labor-intensive in routine use.

Hepatitis C virus particles contain a core antigen (Ag) which encapsidates the viral RNA. The core Ag is present in the serum of infected individuals, probably in both complete virions and RNA-free core protein structures (18). Recently, a highly sensitive and quantitative immunoassay for HCV Ag was developed (the Architect HCV Ag test; Abbott Diagnostics, Rungis, France). It has a cutoff of 3 fmol/liter (0.06 pg/ml). Previous studies using this assay have indicated that (i) the time course of HCV Ag levels is similar to those of HCV RNA for all phases of infection (11, 17) and (ii) the serum concentrations of HCV Ag and RNA are closely correlated (8–10, 12, 17).

However, the HCV Ag assay has not previously been applied to the measurement of intrahepatic HCV Ag levels. The liver is the primary site of HCV replication, and previous work revealed a direct correlation between liver and serum HCV RNA levels (16, 20–22). Here, we sought to investigate the applicability of the assay to quantitation of HCV Ag in paired serum and liver biopsy specimens from treatment-naïve, HCV-infected patients. We also investigated the relationship between the intrahepatic viral load and the serum viral load, as determined by Ag and RNA assays.

We retrospectively evaluated liver biopsy specimens and sera collected from 22 chronically HCV-infected patients and 3 HCV-negative patients. For each patient, a percutaneous liver biopsy specimen and a serum sample were simultaneously collected and stored at −80°C. Epidemiological, clinical, and biochemical data (age, gender, HCV genotype, fibrosis, and alanine aminotransferase levels [IU/liter]) were also recorded (Table 1).

Each sample of frozen liver tissue was weighed and cut into two similar portions. One portion was used for total protein extraction and homogenized in passive lysis buffer (Promega France, Charbonnières, France) on a gentleMACS Dissociator (Miltenyi Biotec SAS, Paris, France). The total protein yield was determined on a NanoDrop spectrophotometer (Thermo Scientific, Courtaboeuf, France). The second portion was used for total RNA extraction. It was dissociated in RNeasy lysis buffer (Qiagen, Courtaboeuf, France), as described above. After centrifugation, total RNA was purified using an RNeasy minikit (Qiagen). The total RNA yield was determined with a spectrophotometer at 260 nm. Total RNA was extracted from serum as described previously (3).

The Architect HCV Ag immunoassay was used for the quantitative determination of HCV Ag in paired liver tissue and sera. The serum concentration of HCV Ag was expressed in pg/ml. Specimens with concentration values of <0.06 pg/ml were considered nonreactive for HCV Ag. For liver tissue, the results were expressed as pg HCV Ag per mg of total extracted protein. HCV RNA in paired liver and serum samples was quantitated with a real-time RT-qPCR assay as previously described (1, 3). Three negative sera and three negative liver biopsy specimens were included as controls.

Statistical analysis was performed using GraphPad Prism5 for Windows (GraphPad Software Inc., La Jolla, CA). The correlation coefficients for serum and liver levels of HCV RNA and Ag were calculated using Spearman's rank test. Intergroup comparisons were performed with the Mann-Whitney U test. A two-sided P value of ≤0.05 was considered statistically significant.

The HCV Ag was present in all infected liver biopsy specimens ($n = 22$), with amounts varying from 1.27 to 3.20 $\log_{10}$ pg/mg total protein (mean: 2.23 ± 0.52) or, relative to the tissue weight, 3.89 ± 0.60 $\log_{10}$ pg/g of liver (range, 2.81 to 5.07). These two parameters showed a strong positive correlation ($r = 0.87$; $P < 0.0001$). The mean hepatic HCV RNA level was 5.81 ± 0.55 $\log_{10}$

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Address correspondence to S. Castelain, sandrine.castelain@u-picardie.fr.

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TABLE 1 Characteristics of the HCV-infected patients

Characteristic	Value
Median age ± SD (yr)	52 ± 13
Gender (no. of males:females)	13:9
Median ALT level ± SD (IU/ml)[a]	65 ± 46
No. with liver fibrosis stage[b]	
F0 or F1	14
F2 or higher	8
No. with HCV genotype	
Type 1	15
Non-type 1	7

[a] The upper limit of normal for alanine aminotransferase (ALT) is 40 IU/ml.
[b] Liver fibrosis was graded according to the METAVIR scoring system.

IU/μg of total RNA (range, 4.72 to 6.74) or, expressed in terms of the amount of tissue, 9.25 ± 0.61 $\log_{10}$ IU/g of liver (range, 7.77 to 10.26). These two parameters were also positively correlated ($r = 0.73$; $P < 0.0001$), suggesting that the quantity of total RNA (and thus total protein) extracted was related to the weight of liver tissue. We further observed that HCV Ag and RNA levels in liver biopsy specimens were highly correlated ($r = 0.83$; $P < 0.0001$) (Fig. 1A). On this basis, a quantitative relationship between HCV RNA and HCV Ag was derived: HCV Ag ($\log_{10}$ pg/g) = 0.8044 × HCV RNA ($\log_{10}$ IU/g) − 3.555. We calculated that 1 pg of HCV Ag per gram was equivalent to approximately 26,276 IU/g (95% confidence interval [CI], 3.8×10^3 to 2.3×10^5 IU/g).

In serum samples, the HCV Ag titer ranged from 0.61 to 2.19 $\log_{10}$ pg/ml (mean, 1.62 ± 0.43). All samples that were positive for HCV Ag ($n = 22$) were also positive for HCV RNA (mean, 6.43 ± 0.58 $\log_{10}$ IU/ml). There was a statistically significant correlation between HCV Ag and HCV RNA levels in sera ($r = 0.90$; $P < 0.0001$) (Fig. 1B). We calculated that 1 pg of HCV Ag per ml is equivalent to 9,755 IU/ml (95% [CI], 3.4×10^3 to 3.1×10^4 IU/ml).

To establish whether the HCV Ag level in liver tissue was related to the HCV Ag level in serum samples, we performed a cor-

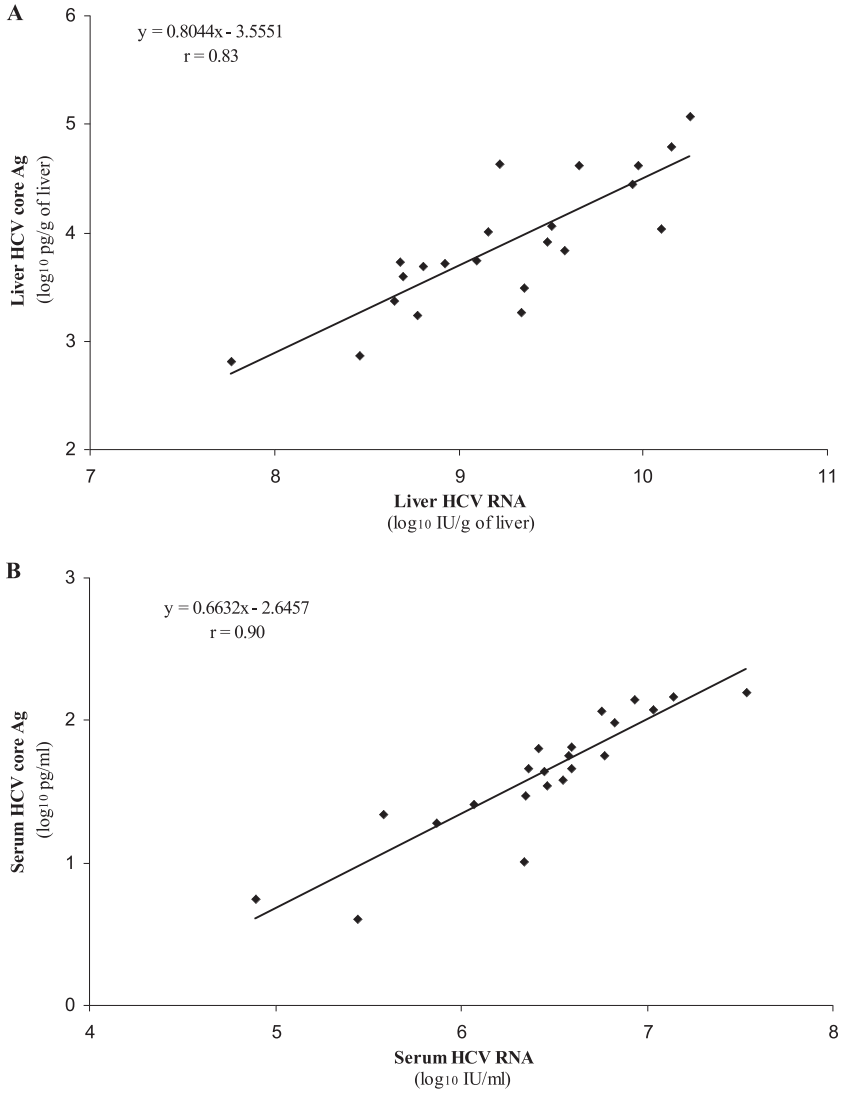

FIG 1 Relationship between the Architect HCV core Ag and HCV RNA concentrations determined in 22 paired samples. (A) Liver biopsy samples; (B) serum samples.

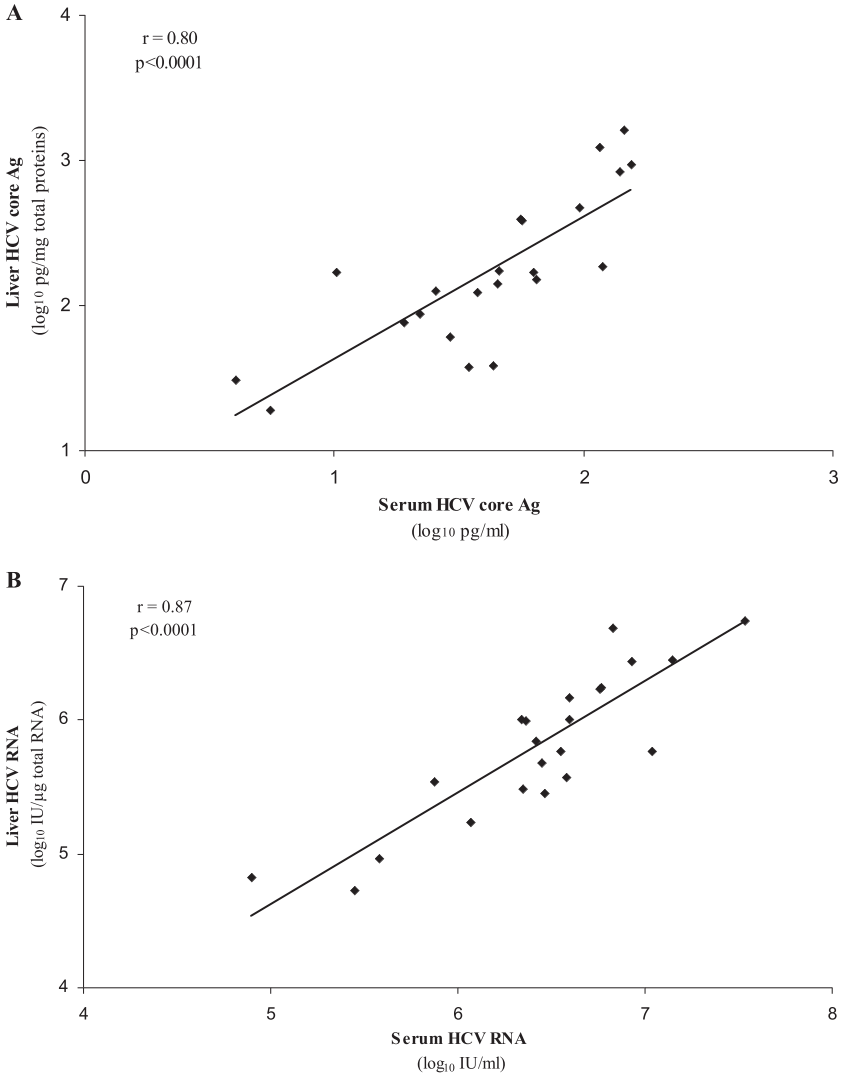

FIG 2 (A) Relationship between HCV core Ag levels in 22 liver-serum pairs, expressed in terms of picograms per ml of serum sample and picograms per milligram of total liver proteins. (B) Correlation between HCV RNA levels in 22 liver-serum sample pairs, expressed in terms of international units per ml of serum sample and international units per microgram of total liver RNA.

relation analysis on the 22 liver-serum pairs. A positive statistically significant correlation was observed ($r = 0.80$; $P < 0.0001$) (Fig. 2A). The same comparison was made for HCV RNA level in liver and in serum. Again, a strong association was found ($r = 0.87$; $P < 0.0001$) (Fig. 2B), suggesting that HCV Ag and HCV RNA are both reliable markers of HCV replication. However, HCV marker levels did not correlate with the aminotransferase activities or fibrosis in the 22 HCV-infected patients (data not shown).

This study evaluated the use of the Architect HCV Ag assay to quantitate HCV Ag in paired serum-liver biopsy specimens from treatment-naïve, chronically infected patients. For the group of infected patients tested here, we found a mean HCV Ag level of 2.23 $\log_{10}$ pg/mg total protein (3.89 $\log_{10}$ pg/g of liver tissue). Both measurements appear to be suitable, since there was a significant correlation between the amounts of liver HCV Ag per milligram of total proteins and per gram of tissue, respectively. Importantly, we observed a strong correlation between concentrations of HCV Ag and HCV RNA in liver biopsy specimens ($r = 0.80$) and in serum ($r = 0.90$). In two previous studies using the same Ag assay and a commercial RT-qPCR assay, the correlation coefficients for concentrations of HCV Ag and HCV RNA in sera were 0.90 ($n = 98$) and 0.94 ($n = 282$), respectively (9, 12). In another study, HCV Ag and RNA levels were more strongly correlated before therapy than after therapy (19). We also observed a statistically significant correlation between hepatic and serum levels of both HCV Ag ($r = 0.80$) and HCV RNA ($r = 0.87$). A number of studies have shown that the HCV viremia in serum mirrors the intrahepatic HCV RNA level (16, 20–22). Here, we have demonstrated that the HCV Ag concentration in serum also mirrors the intrahepatic concentration.

We estimated that 1 pg of total HCV Ag per ml of serum was equivalent to approximately 9,700 IU of HCV RNA. It has already been reported that 1 pg of core Ag per ml is equivalent to approximately 8,000 IU of HCV RNA (2, 18). In theory, 1 pg of virus core should contain 44,000 IU of RNA (13). On this basis, only 22% on average of the core protein measured in our serum samples would

be associated with HCV particles. The remainder may therefore be associated with HCV RNA-free structures (6).

In liver tissue, we demonstrated that 1 pg of total HCV Ag is equivalent to 26,000 IU of HCV RNA per gram of liver. Thus, one can suppose that around two-thirds of the core Ag in liver should be associated with positive-strand RNA (5, 14). The measured levels of core Ag suggest overproduction of the latter by infected hepatocytes and the subsequent release into the blood as RNA-free core structures. Hence, the fact that our observed HCV RNA-to-core Ag ratios in serum and liver were not equivalent suggests the presence of RNA-free core structures secreted by the infected cells and/or generated by *in vivo* degradation in the blood. As is the case for HBsAg, the excess of HCV Ag could function as a decoy for the immune system. However, larger studies including HCV-infected individuals with broader clinical status would be needed to support these data.

In conclusion, HCV Ag and RNA quantities were strongly correlated in both the liver and the serum of chronically infected, treatment-naïve patients. This result demonstrates that serum HCV viremia accurately reflects the amount of virus present in the liver. On the basis of this good correlation, the newly developed, automated HCV Ag assay could be used as an alternative to HCV RNA assays.

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